Proposed Registration Decision PRD2011-24

Bacillus firmus strain I-1582

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Overview

Proposed Registration Decision for *Bacillus firmus* strain I-1582

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Bacillus firmus* GB126 Technical Nematicide and Votivo 240 FS Nematicide, containing the technical grade active ingredient *Bacillus firmus* strain I-1582, to suppress certain soil nematodes in soybean and corn seeds.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

This Overview describes the key points of the evaluation, while the Science Evaluation section provides detailed technical information on the human health, environmental and value assessments of *Bacillus firmus* GB126 Technical Nematicide and Votivo 240 FS Nematicide.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[1] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its proposed conditions of registration. The Act also requires that products have value[2] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (e.g. children) as well as organisms in the environment (e.g. those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticide and Pest Management portion of Health Canada's website at healthcanada.gc.ca/pmra.

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1 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

2 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact."

Before making a final registration decision on *Bacillus firmus* strain I-1582, the PMRA will consider all comments received from the public in response to this consultation document[3]. The PMRA will then publish a Registration Decision[4] on *Bacillus firmus* strain I-1582, which will include the decision, the reasons for it, a summary of comments received on the proposed final registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation section of this consultation document.

What Is *Bacillus firmus* strain I-1582?

Bacillus firmus strain I-1582 is a soil bacterium that degrades the eggs of certain nematode species, most likely through the action of extracellular enzymes. Other mechanisms may be involved in the nematicidal activity of the bacterium.

Health Considerations

Can Approved Uses of *Bacillus firmus* strain I-1582 Affect Human Health?

***Bacillus firmus* strain I-1582 is unlikely to affect your health when Votivo 240 FS Nematicide is used according to the label directions.**

As the end-use product, Votivo 240 FS Nematicide, is a seed treatment, exposure to *Bacillus firmus* strain I-1582 occurs mainly during handling and applying the product.

When assessing health risks, several key factors are considered:

- the microorganism's biological properties (e.g., production of toxic byproducts);
- reports of any adverse incidents;
- its potential to cause disease or toxicity as determined in toxicological studies; and
- the levels to which people may be exposed relative to exposures already encountered in nature to other isolates of this microorganism.

Toxicological studies in laboratory animals describe potential health effects from large doses in order to identify any potential pathogenicity, infectivity and toxicity concerns.

When *Bacillus firmus* strain I-1582 was tested on laboratory animals, there were no signs that it caused any toxicity or disease. Votivo 240 FS Nematicide was mildly irritating to the skin and eyes.

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3 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

4 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

Like all bacteria, *Bacillus firmus* strain I-1582 contain substances that can cause allergic reactions in people who are repeatedly exposed to it at high concentrations. However, these reactions can be avoided if workers and applicators follow label recommendations to minimize or limit exposure to Votivo 240 FS Nematicide.

Residues in Water and Food

Dietary risks from food and water are not of concern.

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a maximum residue limit (MRL) under the *Pest Control Products Act* (PCPA) for the purposes of the adulteration provision of the *Food and Drugs Act* (FDA). Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

Since Votivo 240 FS Nematicide is applied as a seed treatment, residues of *Bacillus firmus* strain I-1582 are not expected to be a concern on corn and soybeans at the time of harvest. As *Bacillus firmus* strains are common in soil, the use of Votivo 240 FS Nematicide is not expected to significantly increase the natural environmental background levels of this microorganism. Also, no adverse effects have been attributed to dietary exposure from natural populations of *Bacillus firmus* strain I-1582, and no adverse effects were observed in the acute oral toxicity study with *Bacillus firmus* strain I-1582. Therefore, the establishment of a maximum residue limit is not required for *Bacillus firmus* strain I-1582. As well, the likelihood of residues contaminating drinking water supplies is negligible to non-existent. Consequently, dietary risks are minimal to non-existent.

Occupational Risks From Handling Votivo 240 FS Nematicide

Occupational risks are not of concern when Votivo 240 FS Nematicide is used according to label directions, which include protective measures.

Workers using Votivo 240 FS Nematicide can come into direct contact with *Bacillus firmus* strain I-1582 on the skin, in the eyes, or by inhalation. For this reason, the label will specify that users exposed to Votivo 240 FS Nematicide must wear personal protective equipment, including a long-sleeved shirt, long pants, shoes plus socks, water-proof gloves, and a dust/mist filtering respirator (MSH/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products.

For bystanders, exposure is expected to be much less than that of workers involved in mixing and application activities and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When Votivo 240 FS Nematicide Is Introduced Into the Environment?

Environmental risks are not of concern.

Following application, *Bacillus firmus* strain I-1582 is likely able to survive in the environment under favourable environmental conditions (i.e., temperature, humidity) but over time populations of *B. firmus* strain I-1582 are expected to return to natural background levels.

Studies were conducted to determine the effects of *B. firmus* strain I-1582 spores on birds, and terrestrial and aquatic invertebrates. These studies showed that *B. firmus* strain I-1582 was not toxic to the Northern Bobwhite quail and honey bees from oral/dietary exposure and no adverse effects were noted for daphnids.

Although avian pulmonary/inhalation/injection, wild mammals, fish, aquatic insects, earthworms, and microorganisms testing were not conducted, adequate information was available to determine that significant adverse effects to these non-target organisms are not expected. There are no published reports of disease associated with *B. firmus* strain I-1582 in birds, wild mammals, fish, aquatic insects and non–arthropod invertebrates. Also, minimal exposure to non-target organisms is anticipated from the proposed use of Votivo 240 FS Nematicide as a seed treatment to suppress root nematodes in soybean and corn seeds.

Value Considerations

What Is the Value of Votivo 240 FS Nematicide?

Votivo 240 FS Nematicide is a microbial seed treatment that suppresses economically important nematode species on corn and soybean.

This product represents the first biological nematicide registered on these field crops. Votivo 240 FS Nematicide constitutes a useful nematode management tool given the increasing nematode populations in Canada as a result of changes in agricultural practices.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures being proposed on the label of Votivo 240 FS Nematicide to address the potential risks identified in this assessment are as follows.

Key Risk-Reduction Measures

Human Health

Although the potential for toxicity is low in individuals exposed to *Bacillus firmus* strain I-1582, sensitization may occur upon repeated exposure to high concentrations of the product. A label statement warning users that the product is a potential sensitizer is required. To minimize exposure to mists generated while handling or applying Votivo 240 FS Nematicide, applicators, mixer/loaders, and handlers will be required to wear personal protective equipment, including a long-sleeved shirt, long pants, shoes plus socks, water-proof gloves, and a dust/mist filtering respirator (MSH/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products

Environment

As a general precaution, the label prohibits the direct application of the product to aquatic habitats (such as lakes, streams and ponds). The label also directs growers to avoid contaminating surface water by disposal of equipment wash waters.

Next Steps

Before making a final registration decision on *Bacillus firmus* strain I-1582, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (contact information on the cover page of this document). The PMRA will then publish a Registration Decision, which will include its decision, the reasons for it, a summary of comments received on the proposed final decision and the Agency's response to these comments.

Other Information

When the PMRA makes its registration decision, it will publish a Registration Decision on *Bacillus firmus* strain I-1582 (based on the Science Evaluation section of this consultation document). In addition, the test data referenced in this consultation document will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

Science Evaluation

Bacillus firmus strain I-1582

1.0 The Active Ingredient, Its Properties and Uses

1.1 Identity of the Active Ingredient

Active microorganism	*Bacillus firmus* strain I-1582
Function	A seed treatment for corn and soybeans to protect roots from soil nematodes
Binomial name	*Bacillus firmus* strain I-1582
Taxonomic designation	
Kingdom	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacilliaceae
Genus	*Bacillus*
Species	*firmus*
Strain	I-1582
Patent Status information	No patents are held by the applicant in Canada.
Minimum purity of active	1.0×10^{11} colony forming units (CFU)/g
Identity of relevant impurities of toxicological, environmental and/or significance.	The TGAI does not contain any impurities or micro contaminants known to be Toxic Substances Management Policy (TSMP) Track 1 substances. The product must meet microbiological contaminants release standards. *Bacillus firmus* strain I-1582 does not produce any known toxins or any other known toxic metabolites.

1.2 Physical and Chemical Properties of the Active Ingredients and End-Use Product

Technical Grade Active Ingredient – *Bacillus firmus* GB126 Technical Nematicide

Colour	Brown
Physical state	Powder
Odour	Earthy
Density	0.44 g/cm^3 mL (loose); 0.55g/ cm^3 (packed)
pH	Not applicable

End-Use – Votivo 240 FS Nematicide

Colour	Brown
Physical state	Liquid suspension
Guarantee	4.8×10^9 CFU/mL
Odour	Petroleum oil like odor
Density	1.1480g/mL at 20.7°C
pH	5.78 at 20.7°C
Viscosity	415 cps with an LV2 spindle at 30 rpm at 20.7°C

1.3 Directions for Use

Votivo 240 FS Nematicide is a seed treatment proposed for early season protection against certain nematode species on corn and soybean. This product is to be applied at 0.042-0.42 mL/1000 seed and diluted in sufficient liquid to achieve uniform distribution. Votivo 240 FS Nematicide is also recommended for use with certain insecticide and fungicide seed treatments.

1.4 Mode of Action

Votivo 240 FS Nematicide contains 21.5% *Bacillus firmus* strain I-1582 as its active ingredient. *B. firmus* strain I-1582 degrades undifferentiated nematode eggs, most likely through the release of extracellular enzymes. Nematode larvae were not shown to be susceptible to *B. firmus* strain I-1582.

2.0 Methods of Analysis

2.1 Methods for Identification of the Microorganism

The active ingredient is identified to the genus level by a series of standard biochemical, morphological and molecular-based tests.

Strain-specific identification of *Bacillus firmus* strain I-1582 can be accomplished through ribotype analysis of the 16S rDNA sequence of strain I-1582.

2.2 Methods for Establishment of Purity of Seed Stock

Frozen cultures (Master Seed stock) of the original isolate are stored at the Institute de Pasteur and at the American Type Culture Collection (ATCC), as well as at Bayer CropScience, and at the site of manufacture of the TGAI. A working stock of the frozen culture is propagated under appropriate conditions, and used to initiate each industrial fermentation run of the TGAI. Prior to propagation, the working stock is plated on suitable media to assure microbial identity and purity.

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The guarantee of the TGAI and end-use product is based on the number of colony forming units per gram or millilitre of product, respectively. Dilutions of the product are plated onto the surface of trypticase soy agar plates and the resulting colonies are counted in order to calculate the viable cell count.

2.4 Methods to Determine and Quantify Residues (Viable or Non-viable) of the Active Microorganism and Relevant Metabolites

Votivo 240 FS Nematicide is applied as a seed treatment. Therefore, no methods to determine and quantify the microbial pest control agent (MPCA) and relevant metabolites are required.

In the event it becomes required to analyze for residues of *Bacillus firmus* strain I-1582 in plants, the ribotype analysis method developed to identify the MPCA in section 2.1 could be used to analyze for the MPCA on food crops.

2.5 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality control procedures used to limit contaminating microorganisms during manufacture of *Bacillus firmus* GB126 Technical Nematicide and Votivo 240 FS Nematicide are acceptable. These procedures include methods to monitor for general microbial contamination as well as for specific pathogens (e.g., *Staphylococcus aureus, Salmonella typhimurium, Shigella flexneri,* and *Escherichia coli*) in the TGAI. Any product that does not meet the applicant's specifications for microbial contamination is destroyed.

During manufacturing, frequent purity checks on agar media and sterilization of the fermentation media are used to limit microbial contamination in the TGAI and end-use product.

The absence of human pathogens and below-threshold levels of contaminants were demonstrated in representative batches of the TGAI using pathogen-specific growth media and general purpose nutrient agar, respectively. Microbe-specific screening methods for *Staphylococcus aureus, Salmonella typhimurium, Shigella flexneri,* and *Escherichia coli* are adequate for detecting and enumerating microbial contaminants of concern.

Release standards for microbial contaminants in the production batches comply with those permitted by the PMRA and are adequate to ensure that the end-use products do not contain unacceptable levels of human and animal disease-causing microorganisms.

A copy of the procedures for screening for general contamination, and for pathogen-specific contamination (i.e., *E. coli*) must be submitted upon finalizing of the procedures.

2.6 Methods to Determine Storage Stability, Shelf-life of the Microorganism

The viability of *Bacillus firmus* strain I-1582 in *Bacillus firmus* GB126 Technical Nematicide was evaluated over 3 years at room temperature (23°C). The submitted storage stability data support a storage period for the TGAI of up to 3 year at room temperature.

For the end-use product, based on interim storage stability test results from a similar formulation of *Bacillus firmus* strain I-1582, and on the nature of the end-use product (i.e., the active ingredient is present as spores), the PMRA can support a storage interval of up to 6 months at room temperature for Votivo 240 FS Nematicide.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the toxicological database for *Bacillus firmus* strain I-1582. The database for *Bacillus firmus* GB126 Technical Nematicide, containing the MPCA *Bacillus firmus* strain I-1582, is complete (see Appendix 1, Table 1), consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity/pathogenicity, acute pulmonary toxicity/pathogenicity, and acute intravenous infectivity) currently required for health hazard assessment purposes. A dermal toxicity/irritation study and a hypersensitivity study were also submitted to address these requirements for the end-use product, Votivo 240 FS Nematicide.

In addition to these required studies, several non-compulsory studies were also conducted. These included a dermal irritation study, and an eye irritation study with *Bacillus firmus* strain I-1582 spores (a substance equivalent to the TGAI), as well as an acute oral toxicity study, an acute inhalation toxicity study, a dermal irritation study, and an eye irritation with the end-use product. A dermal toxicity study with *Bacillus firmus* strain I-1582 spores in nutrient broth was also conducted.

The studies were carried out in accordance with currently accepted international testing protocols and good laboratory practices. The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this microbial pest control agent and product.

As the test substance contains a MPCA that is a spore-forming bacterium, a heat-activation procedure was carried out on the test substance prior to its administration in the acute oral toxicity/pathogenicity study, the acute pulmonary toxicity/pathogenicity study and the IV infectivity study.

In an oral toxicity and infectivity study, groups of fasted, young adult Sprague-Dawley rats (14/sex) were given a single oral dose of *Bacillus firmus* strain I-1582 spores (1.0×10^{10} spores/g) in 0.9% sodium chloride at $>10^8$ CFU/animal in a dosing volume of 0.1 mL. The test substance, *Bacillus firmus* strain I-1582 spores, is equivalent to *Bacillus firmus* GB126 Technical Nematicide. An untreated control group (3/sex) and a shelf control group (2/sex) were conducted concurrently. The animals were observed for a period of up to 14 days with interim scheduled sacrifices on Days 0, 3, and 7. There were no mortalities throughout the study, no signs of toxicological effects, and body weight was largely unaffected by administration of the test substance. The test substance was detected in the fecal samples on Day 1, and had cleared from all tissues/fluids by Day 14 or sooner. Based on the results from this study, there was no evidence of pathogenicity from *Bacillus firmus* strain I-1582 at 1.0×10^8 CFU/animal via the oral route.

In an oral toxicity study, three fasted, young adult female Sprague Dawley rats were given a single oral dose of L1874 (21.5% GB126; potency not reported) at 5000 mg/kg bw. The test substance, L1874, is equivalent to Votivo 240 FS Nematicide. The animals were observed for a period of up to 14 days. There were no mortalities, no signs of gross toxicity, no gross abnormalities upon necropsy, and all animals appeared active and healthy and gained weight during the 14 day observation period. Based on the results from this study, there was no evidence of toxicity from L1874 at 5000 mg/kg bw via the oral route.

In a pulmonary infectivity and toxicity study, groups of fasted, young adult Sprague-Dawley rats (21/sex) were exposed by the intratracheal route to *Bacillus firmus* strain I-1582 spores (1.0×10^{10} spores/g; equivalent to *Bacillus firmus* GB126 Technical Nematicide) in 0.9% sodium chloride at $> 1 \times 10^8$ CFU/animal in a dosing volume of 0.1 mL. An untreated control group (5/sex) and a shelf control group (4/sex) were conducted concurrently. The animals were observed for a period of up to 21 days with interim sacrifices on Days 0, 3, and 7. There were no mortalities throughout the study, no signs of toxicological effects, and body weight was largely unaffected by administration of the test substance. The test substance was detected in the cecum contents on Day 3, but had cleared from all other organs and tissues by Day 14 or sooner. Based on the results of this study, there was no evidence of pathogenicity from *Bacillus firmus* strain I-1582 at 1.0×10^8 CFU/animal via the pulmonary route.

In an acute inhalation toxicity study, groups of young adult Sprague Dawley rats (5/sex) were exposed by the inhalation route to L1874 (potency not reported; equivalent to Votivo 240 FS Nematicide) for 4 hours by nose-only at a concentration of 2.56 mg/L. Animals were observed for 14 days. There were no mortalities during the study. Animals appeared active and healthy and gained weight during the 14-day observation period and there were no gross abnormalities noted upon necropsy. Based on these results, there was no evidence of toxicity from L1874 at 2.56 mg/L via the pulmonary route.

In an acute intravenous/subcutaneous infectivity study, groups of young adult Sprague Dawley rats (21/sex) were injected with *Bacillus firmus* strain I-1582 spores (measured: 1.0×10^{10} CFU/g) in sterile saline at a dose of 1.8×10^{8} CFU/animal in a constant dosing volume of 0.1 mL. An untreated control group (5/sex) was conducted concurrently. Animals were observed for up to 21 days. There were no treatment related clinical signs or necropsy findings, and body weight was largely unaffected. The MPCA had cleared from the cecum contents and liver by Day 14, and from the remaining test organs by Day 21. Based on these results, there is no evidence of pathogenicity from *Bacillus firmus* strain I-1582 at 1.8×10^{8} CFU/animal via the intravenous route.

In a dermal toxicity study, groups of young adult New Zealand White rabbits (5/sex) were dermally exposed to a *Bacillus firmus* strain I-1582 spore suspension (in nutrient broth) at a dose of 5050 mg/kg bw for 24 hours to an area of approximately 10% of the body surface area. Following exposure, the animals were observed for a period of 14 days. There were no treatment related clinical signs, necropsy findings, and no major changes in body weight. Dermal irritation consisted only of very slight to slight erythema on Day 1. Based on these results, there is no evidence of dermal toxicity or irritation from *Bacillus firmus* strain I-1582 spores at 5050 mg/kg bw.

In a dermal toxicity/irritation study, groups of young adult Sprague Dawley rats (5/sex) were dermally exposed to L1874 (21.5% GB126; potency was not reported; equivalent to Votivo 240 FS Nematicide) at a dose of 5000 mg/kg bw for 24 hours to an area of approximately 10% of the body surface area. Following exposure, the animals were observed for a period of 14 days. Erythema and desquamation (redness and peeling) was periodically noted in nine of the ten test animals until Day 12. All signs of irritation had cleared by Day 13. There were no other treatment related clinical signs or necropsy findings for test animals. Aside from a temporary loss of weight in one female on Day 7, all animals gained weight throughout the observation period. Based on these results, mild irritation may occur from dermal exposure to L1874 at 5000 mg/kg bw.

In a primary dermal irritation study, 0.5 mL of Technical SDN (potency not reported; equivalent to *Bacillus firmus* GB126 Technical Nematicide) was applied to a gauze pad and then exposed to a 6-cm^2 body surface area of three young adult female rabbits for 4 hours. Animals were observed for 3 days. Irritation was scored by the method of Draize. Very slight erythema and very slight edema was noted in test animals within one hour of patch removal. All animals were free of all dermal irritation by 48 hours. In this study, Technical SDN is minimally irritating to the skin of rabbits.

In a primary dermal irritation study, three young adult female rabbits were dermally exposed to 0.5 mL of L1874 (21.5% GB126; equivalent to Votivo 240 FS Nematicide) for 4 hours to a 6-cm^2 body surface area. Animals were observed for 3 days. Irritation was scored by the method of Draize. Very slight to well defined erythema and very slight edema was noted in test animals within one hour of patch removal. All animals were free of all dermal irritation by 72 hours. In this study, L1874 is a slight dermal irritant.

In a skin sensitization study (Buehler method), 0.4 mL of the undiluted L1874 (21.5 GB126; potency not reported) was administered topically via a closed patch on the animal's flank to twenty young adult female Guinea Pigs, once a week for three weeks (6-hour exposure) for the induction phase. Irritation was scored at 24- and 48-hours after removal of the patch. Twenty-seven days after the first induction dose, the test animals were administered a challenge dose of 0.4 mL of the undiluted L1874 to the untreated flank (6-hour exposure), and irritation was scored again at 24- and 48-hours after removal of the patch. In the induction phase, two of the twenty test animals exhibited very faint erythema at the 24-hour time point only. Upon administration of the challenge dose, seven of the twenty test animals exhibited very faint erythema at the 24-hour observation. Irritation had cleared from the affected sites by 48 hours. In this study, L1874 is not a dermal sensitizer. Nevertheless, because all microorganisms contain substances that could elicit positive hypersensitivity reactions in humans, *Bacillus firmus* strain I-1582 is considered to be a potential sensitizing agent. Consequently the signals words "POTENTIAL SENSITIZER" are required on the principal display panels of *Bacillus firmus* GB126 Technical Nematicide and Votivo 240 FS Nematicide labels.

In a primary eye irritation study, 0.05g of *Bacillus firmus* strain I-1582 spores (potency not reported; equivalent to *Bacillus firmus* GB126 Technical Nematicide) was instilled into the conjunctival sac of the right eye of three young adult male New Zealand White rabbits. Eyes of treated animals were examined for 4 days and scored for ocular irritation by the method of Draize. One hour after instillation, all three treated eyes exhibited iritis and 'positive' conjunctivitis. The overall incidence and severity of irritation decreased with time and no ocular irritation was noted by Day 4. There were no mortalities in the study, and all animals appeared active and healthy during the study with no signs of gross toxicity, abnormal behavior, or adverse pharmacologic effects other than the noted eye irritation. In this study, *Bacillus firmus* strain I-1582 spores are mildly irritating to the eye. The standard precautionary statement "CAUTION-Eye Irritant" must be included on the technical label, but eye goggles are not considered necessary.

In a primary eye irritation study, 0.1 mL of L1874 (potency not reported; equivalent to Votivo 240 FS Nematicide) was instilled into the conjunctival sac of the right eye of three young adult male New Zealand White rabbits. Eyes of treated animals were examined for 4 days and scored for ocular irritation by the method of Draize. One hour after test substance instillation, two treated eyes exhibited corneal opacity, and all three treated eyes exhibited iritis and 'positive' conjunctivitis. The overall incidence and severity of irritation decreased with time. No ocular irritation was noted by Day 4. There were no mortalities in the study, and all animals appeared active and healthy with no signs of gross toxicity, abnormal behavior, or adverse pharmacologic effects other than the noted eye irritation. In this study, L1874 is mildly irritating to the eye. The standard precautionary statement "CAUTION-Eye Irritant" must be included on the end-use product label, but eye goggles are not considered necessary.

A survey of published literature has revealed no clinical cases of infection from *Bacillus firmus* strain I-1582.

Higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA, and no indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute oral and pulmonary toxicity/infectivity tests.

Within the available scientific literature, there are no reports that suggest *Bacillus firmus* strain I-1582 has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following oral and pulmonary routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *Bacillus firmus* strain I-1582 strain I-1852.

3.2 Occupational/Bystander Exposure and Risk Assessment

3.2.1 Occupational

Votivo 240 FS Nematicide will be applied as water-based slurry to treat corn and soybean seeds using commercial seed treatment equipment. When used according to the proposed label instructions, the potential routes of worker exposure to Votivo 240 FS Nematicide are dermal, pulmonary, and to some extent ocular. However, the PMRA does not expect that the occupational exposure from the proposed use in commercial farms and seed treatment facilities will be of concern on the basis of the low toxicity/pathogenicity profile for *Bacillus firmus* strain I-1582, and on the assumption that the precautionary labelling instructions aimed at minimizing worker exposure are followed by users.

Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, or if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *Bacillus firmus* has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Based on the toxicological profile for *Bacillus firmus* strain I-1582, dermal toxicity from exposure to *Bacillus firmus* strain I-1582 or the end-use product is not expected. Minor dermal irritation, however, (i.e., redness and peeling) could result from dermal exposure to the end-use product. To mitigate the risk of dermal exposure, users exposed to Votivo 240 FS Nematicide will be required to wear appropriate personal protective equipment, including waterproof gloves, long-sleeved shirts, long pants, and shoes plus socks.

Pulmonary exposure may occur when workers are treating the seeds using the commercial mist applicators. Based on the toxicological profile for *Bacillus firmus* strain I-1582, exposure to a large single quantity of the MPCA via the pulmonary route is not of concern.

Based on the results of the eye irritancy study, Votivo 240 FS Nematicide could cause mild ocular irritation on exposure. As the end-use formulation being proposed for registration is a liquid, the potential for ocular exposure is greatest during loading activities. Precautionary statements on the end-use product label warning users of the potential for eye irritation (i.e., Caution: Eye Irritant) will adequately mitigate the risks from exposure.

Although no dermal toxicity and little dermal irritation is expected based on toxicological studies of the MPCA and toxicological characteristics of the formulation ingredients present in the end-use formulation, sensitization may occur upon repeated exposure to high concentrations of the product since the PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions. As all MPCAs are considered potential sensitizers, a label statement warning users that the product is a potential sensitizer is required. To minimize exposure while applying the product to seeds, and handling the treated seeds, mixer/loaders and handlers will be required to wear personal protective equipment, including a long-sleeved shirt, long pants, shoes plus socks, water-proof gloves, and a dust/mist filtering respirator (MSH/NIOSH approval number prefix TC-21C) or a NIOSH approved respirator with any N-95, R-95, P-95 or HE filter for biological products.

3.2.2 Bystander

Inhalation or dermal exposure to the general public is expected to be low based on the proposed seed treatment application of Votivo 240 FS Nematicide on corn and soybean. Overall the PMRA does not expect that bystander exposures will pose an undue risk on the basis of the low toxicity/pathogenicity profile for *Bacillus firmus* strain I-1582 and the related end-use formulation.

The label does not allow applications to turf, residential or recreational areas; therefore, non-occupational dermal exposure and risk to adults, infants and children are low. Because the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

Votivo 240 FS Nematicide is proposed as a commercial seed treatment on corn and soybean seeds using commercial mist or seed treatment equipment.

Based on the seed treatment use of Votivo 240 FS Nematicide, the likelihood of bacteria to remain as residues on the corn and soybeans at harvest is very low. Furthermore, *Bacillus firmus* strain I-1582 strains are common in nature, and the use of Votivo 240 FS Nematicide as a seed treatment is not expected to significantly increase the natural environmental background levels of this microorganism. No adverse effects have been attributed to dietary exposure from natural populations of *Bacillus firmus* strain I-1582. When *Bacillus firmus* strain I-1582 was administered orally to rats, there was no significant toxicity, and no signs of disease were observed. Consequently, higher tier subchronic and chronic dietary exposure studies were not required by PMRA.

As there are no concerns for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children, the PMRA does not require crop residue data on seeds treated with *Bacillus firmus* strain I-1582.

3.3.2 Drinking Water

The likelihood that *Bacillus firmus* strain I-1582 could enter neighbouring aquatic environments as a result of the seed treatment use is negligible. No risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because there were no harmful effects observed in Tier I acute oral toxicity and infectivity testing. The Votivo 240 FS Nematicide label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal. Furthermore, municipal treatment of drinking water is expected to remove the transfer of residues to drinking water. Therefore, potential exposure to *Bacillus firmus* strain I-1582 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculations of acute reference doses (ARDs) and acceptable daily intakes (ADIs) are not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk, if no significant adverse effects (i.e., no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that the MPCA is of low toxicity, is not pathogenic or infective to mammals, and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further factoring of consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of the MPCA, including neurological effects from pre- or post-natal exposures, and cumulative effects on infants and children of the MPCA and other registered micro-organisms that have a common mechanism of toxicity, does not apply to this MPCA. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of this MPCA to human health.

3.4 Maximum Residue Limits

As part of the assessment process prior to the registration of a pesticide, Health Canada must determine whether the consumption of the maximum amount of residues, that are expected to remain on food products when a pesticide is used according to label directions, will not be a concern to human health. This maximum amount of residues expected is then legally established as a MRL under the PCPA for the purposes of the adulteration provision of the FDA. Health Canada sets science-based MRLs to ensure the food Canadians eat is safe.

Since Votivo 240 FS Nematicide is applied as a seed treatment, residues of *Bacillus firmus* strain I-1582 are not expected to be a concern on corn and soybeans at the time of harvest. As *Bacillus firmus* strain I-1582 strains are common in soil, the use of Votivo 240 FS Nematicide is not expected to significantly increase the natural environmental background levels of this microorganism. Also, no adverse effects have been attributed to dietary exposure from natural populations of *Bacillus firmus* strain I-1582, and no adverse effects were observed in the acute oral toxicity study with *Bacillus firmus* strain I-1582. Therefore, the establishment of a maximum residue limit is not required for *Bacillus firmus* strain I-1582.

3.5 Aggregate Exposure

Based on the toxicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty no harm will result from aggregate exposure of residues of *Bacillus firmus* strain I-1582 to the general Canadian population, including infants and children, when the microbial pest control product Votivo 240 FS Nematicide is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information. Furthermore, there have been no adverse effects from exposure to natural populations of *Bacillus firmus* strain I-1582 in the environment. Even if there is an increase in exposure to this microorganism from the uses of Votivo 240 FS Nematicide, there should not be any increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of such residues and other substances that have a common mechanism of toxicity. These considerations included the cumulative effects on infants and children of such residues and other substances with a common mechanism of toxicity.

Besides naturally occurring strains of *Bacillus firmus* strain I-1582 in the environment, the PMRA is not aware of any other microorganisms, or other substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *Bacillus firmus* strain I-1582 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Environmental fate testing is intended to demonstrate whether a MPCA is capable of surviving or replicating in the environment to which it is applied, and could provide an indication of which non–target organisms may be exposed to the MPCA, as well as an indication of the extent of exposure. Environmental fate data are not normally required for Tier I risk assessment purposes and are only triggered if significant toxicological effects in non–target organisms are noted in Tier I testing. Since no significant toxicological effects in non–target organisms are expected from the seed treatment use of Votivo 240 FS Nematicide, no fate data were required to complete the environmental risk assessment of *Bacillus firmus* GB126 Technical and Votivo 240 FS Nematicide.

Bacillus firmus is a gram-positive bacterium that has been isolated from the air, streams, soils, oil wells, oceans and a variety of plant root surfaces. *B. firmus* is ubiquitous in the environment and has been isolated from the soils of several continents including South America, Europe, North America, Africa and Asia. In sandy loam soil in Greece, *B. firmus* can be found at concentrations ranging from 10^5 to 10^8 colony forming units (CFU)/g of dry soil. Symbiotic behaviour of *B. firmus* with bees (fossilized and contemporary) and soil borne root-colonizing microorganisms have also been described in the published literature.

There is no known production of genotoxic, carcinogenic, allergenic, mutagenic or toxic metabolites or antibiotics by *B. firmus*. In addition according to the scientific literature no natural populations of *B. firmus* or its metabolites have been associated with adverse effects in the environment.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

The data and information available on the proposed MPCA and use pattern are sufficient to adequately characterize the risk to the terrestrial environment from the use of the end-use product Votivo 240 FS Nematicide.

In a 30-day acute oral toxicity study, 6 groups (5/group) of 14-day-old Northern Bobwhite quails (*Colinus virginianus*) were administered *B. firmus* spores by gavage at 1.25×10^9 CFU/kg bw per day for a five day period. The attenuated controls (2 groups with 5 birds each), which received heat killed *B. firmus* spores, were also included. There were no treatment-related mortalities or overt signs of toxicity reported for the *B. firmus* treatment groups. No evidence of pathogenicity due to treatment was observed during gross necropsy at test termination and no treatment-related effects on body weight or feed consumption were reported for the study. The acute oral LD_{50} was $>1.25\times10^9$ CFU/kg bw per day. The no-observed effect level (NOEL) of *B. firmus* spores on the Northern Bobwhite quails, based on body weight gain, was 1.25×10^9 CFU/kg bw per day.

In a 24-day dietary toxicity study, 4 groups of adult honey bees *(Apis mellifera)* containing approximately 40 to 60 honey bees each were exposed to spores of *B. firmus* at 10^8 spores/mL of a 30% sucrose solution. Two additional groups (approximately 40 to 60 honey bees each) included: an untreated control substance group, which received only the 30% sucrose solution, and a positive control group, which received a 30% sucrose solution containing 1000 ppm of potassium arsenate. The number of dead bees in each cage was assessed on a daily basis. The positive control produced 100% mortality by Day 1 of the study. On Day 24, the study was terminated because cumulative percent mortality in the untreated control group exceeded 20% (24.52%). No statistically significant differences in mortality were reported between the *B. firmus* at 10^8 spores/mL treatment and the untreated control. No behavioural or morphological abnormalities were observed in bees exposed to the test or control substance treatments. The LC_{50} was $>10^8$ spores/mL of 30% sucrose solution. The no-observed-effect concentration (NOEC) value, based on mortality/sublethal effects, was 10^8 spores/mL.

In addition to the above studies, scientific rationales were submitted to waive testing on avian inhalation, wild mammals, terrestrial plants, and terrestrial arthropods.

The rationales to waive data for effects to the above noted terrestrial non-target organisms were deemed acceptable and were based on the limited exposue to the terrestrial environment expected from the seed treatment use and the lack of effects toward these non-target organisms in the published scientific literature, given the ubiquitous nature of *B. firmus* in the environment.

No studies were submitted to address effects to non–target microorganisms and non–arthropods invertebrates nor were scientific rationales provided to waive the data. However, based on the accounts in the published literature of the ancient association of *B. firmus* with other soil dwelling microorganisms, and that once applied there is not expected to be a sustained increase in the background levels of *B. firmus* strain I-1582 in the terrestrial environment, adverse effects are not expected. Furthermore, as there is no indication that *B. firmus* I-1582 produces any secondary metabolites which contribute to its mode of action, the soil microflora is not expected to be adversely affected by exposure to *B. firmus* strain I-1582 treated seeds. Therefore, the requirement for microorganism testing has been waived

The mode of action of *B. firmus* I-1582 on the target pest is a highly specific and very complex process with spores attaching to the shells of undifferentiated target nematodes eggs or eggs in a very early development stage. Once attached to the egg-shell, *B. firmus* I-1582 cells start to perforate the outer-layer of the nematode eggs probably by the involvement of hydrolytic enzymes. Given the very specific mode of action of the MPCA towards the target pest, and considering the lack of adverse effects in the published literature to other non-arthropod invertebrates from strains of *B. firmus*, adverse effects are not expected. Non–target testing towards non–arthropod invertebrates is therefore waived.

A search was also conducted of the AGRICOLA, TOXLINE, ABSTRACTS, CHEMTOX and PUBMED, databases for the period 1998-2011. In this literature search, *Bacillus firmus*, toxicity, birds, mammals, insects, earthworms and plants, were used as search words. The results were screened for references to genotoxins, carcinogens, allergens, mutagens, toxic metabolites, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with the terrestrial environment. Other than a report that indicates *B. firmus* may act as a control agent against the larvae of the lepidoteran *Atteva fabriciella*; a pest of soft wood trees, no additional papers were found citing potential adverse effects to insects, birds, mammals and plants from *B. firmus* exposure. There is no evidence in the published literature that would indicate genotoxic, carcinogenic, allergenic, mutagenic or toxic metabolites or antibiotics are produced by the MPCA as a mode of action.

The proposed seed treatment is not expected to result in a sustained increase in the background levels of *B. firmus* strain I-1582 in the terrestrial environment. There is also no known relationship reported in the published scientific literature between *B. firmus* strain I-1582 and known terrestrial animal pathogens and the risk posed by the seed treatment use of Votivo 240 FS Nematicide is not expected to present a concern for mammals, birds, terrestrial invertebrates and plants.

Based on all the available information, including data on the effects of *B. firmus* strain I-1582 to non–target organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, insects, plants and non-target microorganisms from the proposed use of Votivo 240 FS Nematicide.

4.2.2 Effects on Aquatic Organisms

The data and information submitted or available on the proposed MPCA and use pattern are sufficient to adequately characterize the risk to the aquatic environment from the use of the end-use product Votivo 240 FS Nematicide.

In a 21-day toxicity study 5 groups of *Daphnia magna* (10/group) were exposed to *B. firmus* strain I-1582 spores (1.38×10^5 CFU/mL) under static renewal conditions. Five groups (10/group) received no test substance and served as controls. Observations of immobility and number of survivors in each test chamber were made daily for 21 days. The test was considered valid as the immobilization for the control groups did not exceed 20% for the duration of the test. A survival rate of 90% was observed in four of the treated groups and 70% in the fifth group. The test substance was not considered to be pathogenic to *Daphnia magna* during 21-day exposure at the maximum hazardous dose. The 21-day LC_{50} was $>1.38\times10^5$ CFU/mL. The NOEC value, based on mortality/sublethal effects, was 1.38×10^5 CFU/mL.

In addition to the above study, scientific rationales were submitted to waive testing on freshwater and marine fishes and aquatic plants.

The rationales to waive data for effects to the above noted aquatic non–target organisms were deemed acceptable and were based on the low likelihood for exposure from run-off from treated seeds and lack of effects toward these non–target organisms in the published scientific literature, given the ubiquitous nature of *B. firmus* in the environment.

A search was also conducted of the AGRICOLA, TOXLINE, ABSTRACTS, CHEMTOX and PUBMED, databases for the period 1998-2011. In this literature search, *Bacillus firmus*, toxicity, fish, aquatic insects and plants, were used as search words. The results were screened for references to genotoxins, carcinogens, allergens, mutagens, toxic metabolites, antibiotics, mycotoxins, mycocins, pathogenicity, environmental fate and interactions with the aquatic environment. No reports were found citing potential adverse effects to fishes and aquatic insects and plants from *B. firmus* exposure. There is no evidence in the published literature that would indicate genotoxic, carcinogenic, allergenic, mutagenic or toxic metabolites or antibiotics are produced by the MPCA as a mode of action.

The proposed seed treatment is expected to result in minimal exposure to the aquatic environment. There is also no known relationship reported in the published scientific literature between *B. firmus* strain I-1582 and known aquatic animal pathogens and the risk posed by the seed treatment use of Votivo 240 FS Nematicide is not expected to present a concern for fishes, and aquatic insects and plants.

5.0 Incident Reports

Since April 26, 2007, registrants have been required by law to report incidents, including adverse effects to health and the environment, to the PMRA within a set time frame. Information on the reporting of incidents can be found on the Pesticides and Pest Management portion of Health Canada's website http:// www.healthcanada.gc.ca/pesticideincident.

As of July 11, 2011, there have been no incidents related to health or the environment reported to the PMRA, nor summarized by the US EPA or the California Department of Pesticide regulation (CalDPR), for products containing *Bacillus firmus* strain I-1582.

Furthermore, as of September 7, 2011, there were no environmental incidents reported in the PMRA Incident reporting database nor in the US EPA's Ecological Incident Information System (EIIS) for products containing *B. firmus* strain I-1582 for use as pesticides, including the US EPA registered product Chancellor, which contains the active ingredient *B. firmus* strain I-1582.

6.0 Value

6.1 Effectiveness Against Pests

6.1.1 Acceptable Efficacy Claims

In-vitro, greenhouse and field studies were provided in support of the proposed claims. Controlled environment studies showed that 1) spores of *B. firmus* strain I-1582 incorporated into the soil suppressed root-knot nematodes, and 2) the end-use product, Votivo 240 FS Nematicide, suppressed reniform nematodes, when applied to cotton seeds.

A total of 29 field trials on soybean (23) and corn (6) were submitted by the applicant. Various nematode species were sampled in these trials. Treatment with Votivo 240 FS Nematicide did not reduce egg numbers or adult nematode populations. Spatial variability of nematode population densities, soil sampling methods and the mode of action of *B. firmus* strain I-1582 likely explain the high variability within treatments and the lack of significant reduction in nematode or egg counts.

In field trials, the addition of Votivo 240 FS Nematicide (0.042-0.42 mL/1000 seed) to other conventional seed treatments generally provided consistent numerical increases in soybean and corn yield. The low-risk status of Votivo 240 FS Nematicide, the lack of registered nematicides on corn and soybean, the increasing nematode populations in Canada and the economic importance of soybean and corn production in Canada were also considered in the evaluation decision.

Based on the nematicidal activity of *B. firmus* strain I-1582 and Votivo 240 FS Nematicide in controlled environment studies, the corn and soybean yield increases resulting from treatment with Votivo 240 FS Nematicide, and the above value considerations, the use of Votivo 240 FS Nematicide is supported for suppression of needle, root-lesion and root-knot nematodes on corn as well as soybean cyst, root-lesion and root-knot nematodes on soybean.

6.2 Adverse effects

Votivo 240 FS Nematicide applied at the proposed rates generally did not reduce germination, emergence and survival of corn and soybean seeds. No phytotoxicity was observed in the trials.

6.3 Economics

Nematodes use their stylet to puncture root cells and extract their content, which can result in reduction of plant vigour, necrotic or stubby root systems, and patches of chlorotic, wilted or stunted plants. Nematode damage has been a resurgent problem in corn and soybean fields. While nematodes were adequately controlled by older insecticide chemistries (e.g. organophosphates), new production practices such as the use of pyrethroid insecticides and transgenic insect-resistant corn varieties has favoured increases in nematode populations in recent years.

Fumigants are currently registered as pre-plant soil treatments for control of plant parasitic nematodes, although their use is not economically justified on corn and soybean given the large hectarage dedicated to these crops. Votivo 240 FS Nematicide is the first biological seed treatment registered for protection against corn and soybean nematodes in Canada.

6.4 Sustainability

6.4.1 Survey of Alternatives

Fumigant active ingredients, such as metam sodium, are registered as pre-plant treatments for control of nematodes on food and fibre crops. Under the Montreal Protocol on Substances that Deplete the Ozone Layer, the manufacture, import and export of methyl bromide has been prohibited since 2005 in Canada, with the exception of specific critical uses. The fumigant 1,3-dichloropropene has also been voluntarily discontinued by the registrant, with the date of last use of December 31, 2011.

6.4.2 Compatibility with Current Management Practices Including Integrated Pest Management

Votivo 240 FS Nematicide has shown to be compatible in tank-mix with fungicide (trifloxystrobin, metalaxyl) and insecticide (imidacloprid) seed treatments. The substantial yield increases achieved with the addition of Votivo 240 FS Nematicide suggest that tank-mixing with conventional seed treatments does not alter the biological functions of *B. firmus* strain I-1582. Votivo 240 FS Nematicide is recommended for use as part of an integrated pest management program.

6.4.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

Based on the multi-site mode of action of *B. firmus* strain I-1582, the risk of nematode resistance development is not a major concern for Votivo 240 FS Nematicide.

6.4.4 Contribution to Risk Reduction and Sustainability

Votivo 240 FS Nematicide is a microbial product that is compatible with certain fungicide and insecticide seed treatments, which indicates that it can be a component of an integrated pest management program. It is effective in suppressing major nematode species on corn and soybean, field crops for which few nematicidal options are available. Votivo 240 FS Nematicide thus represents a useful nematode management tool that may provide substantial yield benefits to farmers.

7.0 Pest Control Product Policy Considerations

7.1 Toxic Substances Management Policy Considerations

The Toxic Substances Management Policy (TSMP) is a federal government policy developed to provide direction on the management of substances of concern that are released into the environment. The TSMP calls for the virtual elimination of Track 1 substances [those that meet all four criteria outlined in the policy, i.e. persistent (in air, soil, water and/or sediment), bio-accumulative, primarily a result of human activity and toxic as defined by the *Canadian Environmental Protection Act*].

Bacillus firmus GB126 Technical Nematicide and Votivo 240 FS Nematicide were assessed in accordance with the PMRA Regulatory Directive DIR99-03[5]

- *Bacillus firmus* GB126 Technical Nematicide does not meet the Track 1 criteria because the active ingredient is an organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products.

- One of the two options for preservatives in end-use product formulation for Votivo 240 FS Nematicide contains micro-contaminants (low levels of dioxins and furans) which are TSMP Track-1 substances. The PMRA's strategy to manage Track 1 contaminants in pest control products is outlined in DIR99-03. The alternative preservative does not meet the Track 1 criteria and as such the registrant will be encouraged to use it in its formulation of Votivo 240 FS Nematicide.

7.2 Formulants and Contaminants of Health or Environmental Concern

During the review process, contaminants in the technical and formulants and contaminants in the end-use products are compared against the *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* maintained in the *Canada Gazette*[6]. The list is used as described in the PMRA Notice of Intent NOI2005-01[7] and is based on existing policies and regulations including: DIR99-03; and DIR2006-02[8] and taking into consideration the Ozone-

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5 Regulatory Directive DIR99-03, The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy

6 *Canada Gazette*, Part II, Volume 139, Number 24, SI/2005-11-30) pages 2641-2643: *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern* and in the order amending this list in the *Canada Gazette*, Part II, Volume 142, Number 13, SI/2008-67 (2008-06-25) pages 1611-1613: *Part I Formulants of Health or Environmental Concern, Part 2 Formulants of Health or Environmental Concern that are Allergens Known to Cause Anaphylactic-Type Reactions and Part 3 Contaminants of Health or Environmental Concern.*

7 Notice of Intent NOI2005-01, *List of Pest Control Product Formulants and Contaminants of Health or Environmental Concern under the New Pest Control Products Act*

8 Regulatory Directive DIR2006-02, *PMRA Formulants Policy*

depleting Substance Regulations, 1998, of the *Canadian Environmental Protection Act* (substances designated under the Montreal Protocol). The PMRA has reached the following conclusions:

- While one of the constituents of a formulant in the end-use product potentially is of concern to the aquatic environment, its presence is considered acceptable given that its relative concentration is 139 µg/L or ppm and the fact that the end-use product is used as a seed treatment which will result in negligible exposure to the aquatic environment.
- *Bacillus firmus* GB126 Technical Nematicide and Votivo 240 FS Nematicide end-use product do not contain any other formulants or contaminants of health or environmental concern identified in the *Canada Gazette*.

The use of formulants in registered pest control products is assessed on an ongoing basis through PMRA formulant initiatives and DIR2006-02.

8.0 Summary

8.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for *Bacillus firmus* GB126 Technical Nematicide and Votivo 240 FS Nematicide were deemed adequate to assess their potential human health and environmental risks. The TGAI was characterized and the microbial contaminant specifications were supported by the analyses of a sufficient number of batches.

Storage stability data for the TGAI were sufficient to support a shelf life of 3 year at 23°C, and based on the nature of the end-use product (i.e., spores), the end-use product a storage interval of up to 6 months can be supported for Votivo 240 FS Nematicide.

8.2 Human Health and Safety

The acute toxicity and infectivity studies submitted in support of *Bacillus firmus* strain I-1582 were determined to be sufficiently complete to permit a decision on registration. *Bacillus firmus* strain I-1582 is of low toxicity and is not pathogenic by the oral and pulmonary route, and is not a dermal irritant. There was also no evidence of pathogenicity from *Bacillus firmus* strain I-1582 via the intravenous route of exposure. Votive 240 FS Nematicide is considered a mild dermal irritant. As *Bacillus firmus* strain I-1582 spores and Votivo 240 FS Nematicide were mild ocular irritants, the standard precautionary statement "CAUTION-Eye Irritant" must be included on both the technical and end-use product label, but eye goggles are not considered necessary.

When used according to the proposed label instructions, the potential routes of worker exposure to Votivo 240 FS Nematicide are dermal, pulmonary, and to some extent ocular. However, the PMRA does not expect that the occupational exposure from the proposed use in commercial farms and seed treatment facilities will be of concern on the basis of the low toxicity/pathogenicity profile for *Bacillus firmus* strain I-1582, and on the assumption that the precautionary labelling instructions aimed at minimizing worker exposure are adhered to by users.

While *Bacillus firmus* strain I-1582 has the potential to be a sensitizing agent, inhalation and dermal exposure is not a concern if the required dust/mist filtering respirator/mask and appropriate PPE stipulated on the end-use product label is worn by persons involved in loading Votivo 240FS Nematicide. Furthermore, precautionary labelling will alert users of the potential sensitization hazard of the end-use products.

Based on the seed treatment use of Votivo 240 FS Nematicide, the likelihood of bacteria to remain as residues on the corn and soybeans at harvest is very low. Furthermore, since *Bacillus firmus* strains are common in nature, a seed treatment use is not expected to significantly increase the natural environmental background levels of this microorganism. No adverse effects have been attributed to dietary exposure from natural populations of *Bacillus firmus* strain I-1582, and when *Bacillus firmus* strain I-1582 was administered orally to rats, there was no significant toxicity and no signs of disease were observed. Consequently, higher tier subchronic and chronic dietary exposure studies were not required by PMRA. For these reasons, the PMRA does not require crop residue data on seeds treated with *Bacillus firmus* strain I-1582, and the establishment of a maximum residue limit is not required for *Bacillus firmus* strain I-1582.

For the same reasons, no risks are expected from exposure to this microorganism via drinking water. The Votivo 240 FS Nematicide label instructs users not to contaminate irrigation or drinking water supplies or aquatic habitats through equipment cleaning or waste disposal, and municipal treatment of drinking water is expected to further remove the transfer of residues to drinking water.

8.3 Environmental Risk

The environmental fate studies, non-target studies organism testing, scientific rationales and supporting published scientific literature submitted in support of *B. firmus* strain I-1582 were determined to be sufficiently complete to permit a decision on registration. The use of Votivo 240 FS Nematicide containing *B. firmus* strain I-1582 is not expected to pose a risk to birds, mammals, arthropods, fish, and plants.

No additional studies were required to address the environmental fate and behaviour of *B. firmus* strain I-1582. Environmental fate data are higher tier requirements and are not normally required in the absence of significant toxicological effects in non-target organisms in Tier I testing. Environmental exposure to *B. firmus* strain I-1582 is expected to be minimal given that the use of Votivo 240 FS Nematicide is limited to seed treatment.

As a general precaution, the Votivo 240 FS Nematicide label prohibits its direct application to aquatic habitats (such as lakes, streams and ponds). The label also directs users to avoid contaminating surface water by disposal of equipment wash waters.

8.4 Value

The data submitted to register Votivo 240 FS Nematicide are adequate to support the following claims:

- suppression of needle, root-lesion and root-knot nematodes on corn
- suppression of soybean cyst, root-lesion and root-knot nematodes on soybean

8.5 Unsupported Uses

The claim of suppression of sting, dagger, spiral, stunt, stubby root and lance nematodes on corn is not supported, as 1) lance and sting nematodes were not recorded in any of the submitted field trials, 2) above threshold populations of spiral and stunt nematodes did not impact corn yield, and 3) very low dagger and stubby-root nematode pressure was noted in field trials.

9.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act* and Regulations, is proposing full registration for the sale and use of *Bacillus firmus* GB126 Technical Nematicide and Votivo 240 FS Nematicide, containing the technical grade active ingredient *Bacillus firmus* strain I-1582, to suppress certain soil nematodes in soybean and corn seeds.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

µg	micrograms
ADI	acceptable daily intake
ARD	acute reference dose
ATCC	American Type Culture Collection
bw	body weight
CFU	colony forming unit
cm	centimetres
cps	centipoise
EIIS	Ecological Incident Information System
FDA	*Food and Drugs Act*
g	gram
kg	kilogram
IV	intravenous
L	litre
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
mg	milligram
mL	millilitre
MAS	maximum average score
MCPA	microbial pest control agent
MRL	maximum residue limit
NIOSH	National Institute of Occupational Safety and Health
NOEC	no observed effect concentration
NOEL	no observed effect level
PCPA	*Pest Control Products Act*
PMRA	Pest Management Regulatory Agency
PPE	personal protective equipment
ppm	parts per million
rDNA	ribosomal deoxyribonucleic acid
rpm	revolutions per minute
TGAI	technical grade active ingredient
TSMP	Toxic Substances Management Policy
US EPA	United States Environmental Protection Agency

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Bacillus firmus* strain I-1582 and its associated end-use product (Votivo 240 FS Nematicide)

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus firmus* strain I-1582				
Acute Oral Toxicity/ Pathogenicity	Rat-Sprague Dawley 14/sex, $>10^8$ CFU of *Bacillus firmus* strain I-1582 spores in 0.9% sodium chloride/animal; interim sacrifices (3/sex) on Day 0, 3, and 7 Untreated control group: 3/sex; Shelf control group: 2/sex 14-day observation period	LD_{50} (male; female) $>10^8$ CFU/animal	Spores were heat-shocked prior to dosing. No mortalities or effect on body weight gain and no clinical signs of treatment related toxicity, infectivity or pathogenicity. The test substance was initially detected in the feces, brain, blood, cecum, kidneys, liver, lungs, lymph nodes and spleen but had cleared from all tissues/organs by Day 14 or sooner. No gross findings observed at necropsy. **NON-TOXIC, NOT INFECTIVE** **ACCEPTABLE**	PMRA 1972922

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus firmus* strain I-1582				
Acute Oral Toxicity	Rat-Sprague Dawley; 3 female, 5000 mg of L1874/kg bw (potency not reported) 14-day observation period	LD_{50} (female) > 5000 mg/kg bw	No mortalities or effect on body weight gain and no clinical signs of toxicity. No gross findings observed at necropsy. **NON-TOXIC** **ACCEPTABLE**	PMRA 1975423

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus firmus* strain I-1582				
Acute Pulmonary Toxicity and Infectivity	Rat-Sprague Dawley 21/sex, > 10^8 CFU of *Bacillus firmus* strain I-1582 spores in 0.9% sodium chloride/animal; interim sacrifices (3/sex) on Day 0,3, 7, and 14. Untreated control group: 5/sex; Shelf control group: 4/sex 21-day observation period	LD_{50} (male; female) > 10^8 CFU/animal	Spores were heat-shocked prior to dosing. No mortalities and no clinical signs of treatment related toxicity, infectivity or pathogenicity. Body weight was largely unaffected. The test substance was initially detected in the brain, blood, cecum, kidneys, liver, lungs, lymph nodes and spleen but had cleared from all tissues/organs by Day 14 or sooner. No gross findings observed at necropsy. **NON-TOXIC, NON-INFECTIVE** **ACCEPTABLE**	PMRA 1972923
Acute Inhalation Toxicity	Rat-Sprague Dawley 5/sex, > 2.56 mg of L1874/L (potency not reported), 4-hour nose-only exposure 14-day observation period	LC_{50} (male; female) > 2.56 mg/L	No mortalities, no effect on body weight gain and no clinical signs of toxicity. Short-lived (D10–12) nasal discharge in one animal. No gross findings observed at necropsy. **LOW TOXICITY** **ACCEPTABLE**	PMRA 1975422

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus firmus* strain I-1582				
Intravenous Injection Infectivity	Rat-Sprague Dawley 21/sex, 1.8×10^8 CFU *Bacillus firmus* strain I-1582 spores in saline/animal Untreated control group: 5/sex 21-day observation period	LD_{50} (male; female) $> 1.8 \times 10^8$ CFU/animal	Spores were heat-shocked prior to dosing. No mortalities, clinical signs of toxicity or gross lesions were observed in any of the test animals. Body weight gain was largely unaffected. The test substance was initially detected in the brain, blood, cecum, kidneys, liver, lungs, lymph nodes and spleen, but had cleared from all organs/tissues by Day 21 or sooner. **NOT INFECTIVE** **ACCEPTABLE**	PMRA 1972924
Acute Dermal Toxicity/ Irritation	Rabbit-New Zealand White, 5/sex: 5050 mg of *Bacillus firmus* spores suspended in nutrient broth/kg bw (at 10^8 CFU *Bacillus firmus* strain I-1582 strain) 24 hour exposure to 10% body surface area 14-day observation period	LD_{50} (male; female) >5050 mg	No mortalities, no treatment-related clinical signs, no necropsy findings and no major changes in body weight. Very slight erythema on Day 1 only. **LOW TOXICITY** **NOT A DERMAL IRRITANT** **ACCEPTABLE**	PMRA 1972926

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus firmus* strain I-1582				
Acute Dermal Toxicity/ Irritation	Rat-Sprague Dawley, 5/sex, 5000 mg of L1874/kg bw (21.5% *B. firmus* strain I-1584; potency not measured) 24-hour exposure to 10% body surface area 14-day observation period	LD_{50} (male; female) >5000 mg	No mortality. No gross abnormalities noted upon necropsy. Erythema and desquamation noted for 3–9 days in some animals. All animals were clear of irritation by Day 13 or sooner. Temporary weight loss in 1 female (Day 7 only) **LOW TOXICITY** **MILD DERMAL IRRITANT** **ACCEPTABLE**	PMRA 1975417
Acute Dermal Irritation	Rabbit-New Zealand White, 3 females: 0. 5 mL of *Bacillus firmus* spores (potency not measured) Test substance was applied to gauze and then to the skin of rabbits, rather than directly to the rabbit skin. 4 hour exposure to a 6 cm^2 body surface area 3-day observation period.	MAS (female)= 0.33	No mortalities. Very slight erythema and very slight edema noted within 1 hour of removal of patch. All animals were free of irritation by 48 hours. **MINIMAL DERMAL IRRITATANT** **ACCEPTABLE**	PMRA 1972928 (DER= PMRA 2028542)

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus firmus* strain I-1582				
Acute Dermal Irritation	Rabbit-New Zealand White, 3 females 0. 5 mL of L1874/kg body weight (21.5% *B. firmus* strain I-1582; potency not measured) 4 hour exposure to a 6 cm^2 body surface area 3-day observation period	MAS (female)= 0.56	Very slight to well-defined erythema and very slight edema noted within 1 hour of removal of patch. All animals were free of irritation by 72 hours. **SLIGHTLY IRRITATING** **ACCEPTABLE**	PMRA 1975418
Eye Irritation	Rabbit- New Zealand White, 3 males: 0.05 g of *B. firmus* strain I-1582 spores in one eye of each rabbit (potency not reported) 4-day observation period An ocular anesthetic was instilled into the eyes prior to administration of the test substance.	MAS (male)= 7.6	Iritis with conjunctivitis occurred in 3 eyes. All irritation cleared by Day 4. Although the MAS classifies the test substance as Non- to Minimally irritating, due to irritation (redness) still present at 72-hours, the test substance should be classified as Mildly Irritating. **MILD EYE IRRITANT** **ACCEPTABLE**	PMRA 1972961

Study Type	Species, Strain, and Doses	Results	Significant Effects and Comments	Reference(s)
Acute Toxicity/Infectivity of *Bacillus firmus* strain I-1582				
Eye Irritation	Rabbit- New Zealand White, 3 males: 0.1mL of L1874 in one eye of each rabbit (potency not reported). 4-day observation period An ocular anesthetic was instilled into the eyes prior to administration of the test substance.	MAS (male)= 6.2	Corneal opacity (2 eyes) and iritis with conjunctivitis (3 eyes). All irritation cleared by Day 4. Although the MAS would classify the test substance as Non- to Minimally irritating, due to irritation (redness) still present at 72-hours, the test substance should be classified as Mildly Irritating. **MILD EYE IRRITANT** **ACCEPTABLE**	PMRA 1975424
Hypersensitivity testing- Buehler method	Guinea Pig; 20 females Induction: 0.4 mL of undiluted L1874 (potency not reported) administered topically once a week for 3 weeks (6 hour exposure). Challenge: 27 days after the first induction, 0.4 mL of undiluted L1874 to the untreated flank (6 hour exposure). Positive control: alpha-Hexylcinnamaldehyde Technical	Not a dermal sensitiser	Induction: 24-hours after removal of the test substance, 2/20 animals exhibited very faint erythema. No irritation at 4-hour. Challenge: 7/20 animals exhibited very slight erythema at the 24-hour timepoint. Irritation had cleared from the affected sites by 48-hours. **NOT A DERMAL SENSITIZER** **ACCEPTABLE**	PMRA 1975419

The test substance L-1874 is identical to the end-use product, Votivo 240FS Nematicide.
MAS= Maximum Average Score: mean irritation score from 24h + 48h + 72h time points only

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Terrestrial Organisms				
Vertebrates				
Birds (Northern Bobwhite quail)	Avian oral–Laboratory study	Six groups of birds (5/group) gavaged with 1.25×10^9 CFU *Bacillus firmus*/kg bw per day for a five day period. Two control (attenuated) groups (5/group) received heat killed *B. firmus* spores.	No treatment related mortalities or overt signs of toxicity were reported for the *B. firmus* treatment groups. No evidence of pathogenicity observed at gross necropsy. No treatment-related effects on body weight or feed consumption reported for the study. LD_{50} >1.25×10^9 CFU /kg bw NOEL 1.25×10^9 CFU/kg bw **ACCEPTABLE**	PMRA 1972937
	Avian - Inhalation	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for pulmonary exposure to avian species from the proposed use of Votivo 240 FS Nematicide as a seed treatment. No adverse effects to birds were reported in the published scientific literature. **WAIVER RATIONALE ACCEPTED**		PMRA 1972939
Wild Mammals	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure to wild mammals from the proposed use of Votivo 240 FS Nematicide as a seed treatment. No adverse effects to mammals were found in the published scientific literature. In addition, no pathogenicity was observed in acute mammalian toxicity and infectivity testing (see Section 3.1) **WAIVER RATIONALE ACCEPTED**			PMRA 1972939
Invertebrates				
Arthropods				
Terrestrial Arthropods	Honeybee (*Apis mellifera*)	Four groups (40 to 60 bees per group) exposed to spores of *B. firmus* 10^8spores/mL of a 30% sucrose solution A negative control group (40 to 60 bees) received just the 30% solution	No statistically significant differences in mortality were reported between the *B. firmus* at 10^8 CFU/mL treatments and the untreated controls. All positive controls were dead by Day 1 confirming the validity of the exposure system. No behavioural or morphological abnormalities were observed in bees exposed	PMRA 1972941

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
		A positive control group (40 to 60 bees) received 30% sucrose solution with 1000 ppm potassium arsenate	to the test or control substance treatments. The 24-day LC_{50} >10^8 CFU/mL of 30% sucrose solution. NOEC 10^8 CFU/mL. **ACCEPTABLE**	
	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure to terrestrial arthropods from the proposed use of Votivo 240 FS Nematicide as a seed treatment. Other than a report that indicates *B. firmus* may act as a control agent against the larvae of the lepidoteran, *Atteva fabriciella*; a pest of soft wood trees, no additional papers were found citing potential adverse effects to insects from *B. firmus* exposure. **WAIVER RATIONALE ACCEPTED**			PMRA 1072940
Non-arthropods				
Terrestrial Non-Arthropod Invertebrates	A request to waive the requirement for these test data was not submitted. However, based on the properties of the MPCA and the lack of adverse effects to terrestrial non-arthropod invertebrates in the published scientific literature, testing has been waived.			
Microorganisms				
	A request to waive the requirement for test data was not submitted. However, given the ubiquitous nature of the MPCA and that its use as a seed treatment it not expected to result in sustained populations of *Bacillus firmus* strain I-1582 above background levels, adverse effects to the non–target microorganisms is not expected. Therefore, testing has been waived.			
Plants				
Plants	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure to terrestrial plants from the proposed use of Votivo 240 FS Nematicide as a seed treatment. *Bacillus firmus* strain I-1582 is not a plant pathogen and no adverse effects to plants were reported in the published scientific literature. **WAIVER RATIONALE ACCEPTED**			PMRA 1072940
Aquatic Organisms				
Vertebrates				
Fish	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure to fish from the proposed use of Votivo 240 FS Nematicide as a seed treatment. *Bacillus firmus* strain I-1582 is not a fish pathogen and no adverse effects to freshwater and marine fishes were found in the published scientific literature. **WAIVER RATIONALE ACCEPTED**			PMRA 1972939 and 1072940

Organism	Exposure	Protocol	Significant Effect, Comments	Reference
Invertebrates				
Aquatic Arthropods and Non-Arthropod Invertebrates	*Daphnia magna* 21-day static renewal	Five groups of daphnids (10 /group) were exposed to *B. firmus* strain I-1582 of 1.38×10^5 CFU/mL under static renewal conditions. Five control groups of daphnids (10/ group) received no test substance Observations of immobility and survival rates in each test chamber were recorded daily.	Immobilization for the control did not exceed 20% for the duration of the test. Survival rates of 90% were reported for four of the treated groups and 70% survival rate was observed in the fifth group. The test substance was not considered pathogenic to *Daphnia magna* during 21-day exposure at the maximum hazardous dose. The 21-day LC_{50} was $>1.38\times10^5$ CFU/mL. NOEC $>1.38\times10^5$ CFU/mL. **ACCEPTABLE**	PMRA 1972946
Plants				
Aquatic Plants	A request to waive the requirement for test data was submitted based on the properties of the MPCA and the limited potential for exposure to aquatic plants from the proposed use of Votivo 240 FS Nematicide as a seed treatment. *Bacillus firmus* strain I-1582 is not a plant pathogen and no adverse effects to aquatic or terrestrial plants were found in the published scientific literature. **WAIVER RATIONALE ACCEPTED**			PMRA 1972934

Table 3 Votivo 240 FS Nematicide Use (label) Claims Proposed by Applicant and Whether Acceptable or Unsupported

Proposed claim	Supported / Unsupported
Early-season protection on corn at 0.042-0.42 mL/1000 seed against: • needle nematodes (*Longidorus* spp.) • root-lesion nematodes (*Pratylenchus* spp.) • root-knot nematodes (*Meloidogyne* spp.)	Supported for suppression.
Early-season protection on corn at 0.042-0.42 mL/1000 seed against: • sting nematodes (*Belonolaimus* spp.) • dagger nematodes (*Xiphinema* spp.) • spiral nematodes (*Helicotylenchus* spp.) • stunt nematodes (*Tylenchorhynchus* spp.) • stubby root nematodes (*Paratrichodorus* spp.) lance nematodes (*Hoplolaimus* spp.)	Not supported. The nematicidal activity of Votivo 240 FS Nematicide has not been demonstrated on these nematode species. Lance and sting nematodes were not recorded in any of the submitted field trials. Above threshold populations of spiral and stunt nematodes did not impact corn yield. Very low dagger and stubby root nematode pressure was noted in field trials.
Early-season protection on soybean at 0.042-0.42 mL/1000 seed against: • soybean cyst nematodes (*Heterodera glycines*) • root-lesion nematodes (*Pratylenchus* spp.) • root-knot nematodes (*Meloidogyne* spp.)	Supported for suppression.

References

A. List of Studies/Information Submitted by Registrant

1.0 Chemistry
2.0 Human and Animal Health

3.0 Environment

4.0 Value

B. Additional Information Considered

i) Published Information

1.0 Methods of Analysis

ii) Foreign reviews
1.0 Methods of Analysis
2.0 Human and Animal Health